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(54) **INHIBITEURS SELECTIFS DE LA PHOSPHODIESTERASE 9A EN TANT QUE MEDICAMENTS POUR
AMELIORER DES PROCESSUS COGNITIFS**

(54) **SELECTIVE PHOSPHODIESTERASE 9A INHIBITORS AS MEDICAMENTS FOR IMPROVING COGNITIVE
PROCESSES**

(57)

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(57) **Abrégé/Abstract:**

The invention relates to the use of selective phosphodiesterase 9A (PDE9A) inhibitors for producing medicaments for improving perception, powers of concentration, cognitive processes, learning capacity and/or memory retention.



Selective PDE9A inhibitors as medicaments for improving cognitive processes

A b s t r a c t

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Selective phosphodiesterase 9A inhibitors as medicaments for improving cognitive processes

The invention relates to the use of selective phosphodiesterase 9A (PDE-9A)
5 inhibitors for producing medicaments for improving perception, concentration, learning and/or memory.

The cellular activation of adenylate cyclases and guanylate cyclases effects cycli-
zation respectively of ATP and GTP to 5'-3'cyclic adenosine monophosphate
10 (cAMP) and 5'-3' cyclic guanosine monophosphate (cGMP). These cyclic nucleo-
tides (cAMP and cGMP) are important second messengers and therefore play a
central role in cellular signal transduction cascades. Each of them reactivates inter
alia, but not exclusively, protein kinases. The protein kinase activated by cAMP is
called protein kinase A (PKA), and the protein kinase activated by cGMP is called
15 protein kinase G (PKG). Activated PKA and PKG are able in turn to phosphorylate a
number of cellular effector proteins (e.g. ion channels, G-protein-coupled receptors,
structural proteins). It is possible in this way for the second messengers cAMP and
cGMP to control a wide variety of physiological processes in a wide variety of
organs. However, the cyclic nucleotides are also able to act directly on effector
20 molecules. Thus, it is known, for example, that cGMP is able to act directly on ion
channels and thus is able to influence the cellular ion concentration (review in: Wei
et al., *Prog. Neurobiol.*, 1998, 56: 37-64). The phosphodiesterases (PDE) are a
control mechanism for controlling the activity of cAMP and cGMP and thus in turn
these physiological processes. PDEs hydrolyze the cyclic monophosphates to the
25 inactive monophosphates AMP and GMP. At least 21 PDE genes have now been
described (*Exp. Opin. Investig. Drugs* 2000, 9, 1354-3784). These 21 PDE genes can
be divided on the basis of their sequence homology into 11 PDE families (for
proposed nomenclature, see <http://depts.washington.edu/pde/Nomenclature.html>).
Individual PDE genes within a family are differentiated by letters (e.g. PDE1A and
30 PDE1B). If different splice variants within a gene also occur, this is then indicated by
an additional numbering after the letters (e.g. PDE1A1).

Figures:

Fig. 1: Intracellular cGMP concentration in primary rat (E18) cortex cultures after treatment with Example 2. The cells were incubated with Example 2 in the stated concentrations for 20 min. The medium control indicates the cGMP level in untreated cells. The DMSO content was 0.2% in the medium controls and in the substance-treated cells. The cGMP concentration is plotted in fmol/150 000 cells.

Fig. 2: Effect of Example 2 (10 μ M) on the potentiation of the focal excitatory postsynaptic potential (fEPSP) after weak tetanus. Example 2 significantly augmented the increase in the fEPSP when it was flushed in for 10 minutes before to 30 minutes after a weak tetanus (closed symbols). The control was treated with 0.01% DMSO (open symbols). *: $p < 0.05$; analysis of variants with the factors dosage (0 and 10 μ M) and test time with the factor measurement time.

Fig. 3: Effect of Example 2 on the percentage reduction (trial 2/trial 1) in the interaction time in the social recognition test (average + S.E.M.). The animals received intraperitoneal injections of vehicle (10% ethanol, 20% Solutol, 70% physiological saline) or 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg or 3.0 mg/kg of Example 2 immediately after the first meeting (trial 1). Statistical analysis: * $p < 0.05$.

PDE9A - Discovery, properties, distribution

Human PDE9A (GenBank/EMBL accession number NM_002606, cDNA sequence see Sequence Listing, SEQ ID NO:1) was cloned and sequenced in 1998. The amino acid identity with other PDEs does not exceed 34% (PDE8A) and is never less than 28% (PDE5A). With a Michaelis-Menten constant (K_m) of 170 nM, PDE9A has high affinity for cGMP. In addition, PDE9A is selective for cGMP (K_m for cAMP = 230 μ M). PDE9A has no cGMP binding domain (GAF domain) which would suggest allosteric enzyme regulation by cGMP. It was shown in a Western blot analysis that PDE9A is expressed in humans inter alia in testes, brain, small intestine, skeletal muscle, heart, lung, thymus and spleen. The highest expression was found in the brain, small intestine, heart and spleen (Fisher et al., *J. Biol. Chem.*, 1998, 273 (25): 15559-15564). The gene for human PDE9A is located on chromosome 21q22.3 and comprises 21 exons. To date, 20 alternative splice variants of PDE9A have been

identified (Guipponi et al., *Hum. Genet.*, 1998, 103: 386-392; Rentero et al., *Biochem. Biophys. Res. Commun.*, 2003, 301: 686-692). Classical PDE inhibitors do not inhibit human PDE9A. Thus, IBMX, dipyridamole, SKF94120, rolipram and vinpocetine show no inhibition on the isolated enzyme in concentrations of up to 100
 5 μM . An IC_{50} of 35 μM has been demonstrated for zaprinast (Fisher et al., *J. Biol. Chem.*, 1998, 273 (25): 15559-15564).

Murine PDE9A was cloned and sequenced in 1998 by Soderling et al. (*J. Biol. Chem.*, 1998, 273 (19): 15553-15558). This has, like the human form, high affinity for cGMP with a K_m of 70 nM. Particularly high expression was found in the mouse
 10 kidney, brain, lung and heart. Murine PDE9A is not inhibited by IBMX in concentrations below 200 μM either; the IC_{50} for zaprinast is 29 μM (Soderling et al., *J. Biol. Chem.*, 1998, 273 (19): 15553-15558). It has been found that PDE9A is strongly expressed in some regions of the rat brain. These include olfactory bulb, hippocampus, cortex, basal ganglia and basal forebrain (Andreeva et al., *J. Neurosci.*,
 15 2001, 21 (22): 9068-9076). The hippocampus, cortex and basal forebrain in particular play an important role in learning and memory processes.

PDE9A is distinguished by having particularly high affinity for cGMP. The K_m for cGMP is 170 μM (Fisher et al., *J. Biol. Chem.*, 1998, 273 (25): 15559 - 15564). PDE9A is therefore active even at low physiological concentrations, in contrast to
 20 PDE2A ($K_m = 10 \mu\text{M}$; Martins et al., *J. Biol. Chem.*, 1982, 257: 1973-1979), PDE5A ($K_m = 4 \mu\text{M}$; Francis et al., *J. Biol. Chem.*, 1980, 255: 620-626), PDE6A ($K_m = 17 \mu\text{M}$; Gillespie and Beavo, *J. Biol. Chem.*, 1988, 263 (17): 8133-8141) and PDE11A ($K_m = 0.52 \mu\text{M}$; Fawcett et al., *Proc. Nat. Acad. Sci.*, 2000, 97 (7): 3702-3707). In contrast to PDE2A (Murashima et al., *Biochemistry*, 1990, 29: 5285-5292),
 25 the catalytic activity of PDE9A is not increased by cGMP because it has no GAF domain (Beavo et al., *Current Opinion in Cell Biology*, 2000, 12: 174-179). PDE9A inhibitors therefore lead to an increase in the baseline cGMP concentration (see Figure 1). This increase in the baseline cGMP concentration surprisingly led to an improvement in learning and memory in the social recognition test.

30 It has surprisingly been demonstrated that PDE9A inhibitors have an effect on the function of the central nervous system. It has now been found in particular that

selective PDE9A inhibitors are suitable for producing medicaments for improving perception, concentration, learning or memory.

A PDE9A inhibitor for the purposes of the invention is a compound which inhibits
5 human PDE9A under the conditions indicated below with an IC_{50} of less than $10\text{ }\mu\text{M}$, preferably less than $1\text{ }\mu\text{M}$.

A selective PDE9A inhibitor for the purposes of the invention is a compound which inhibits human PDE9A under the conditions indicated below more strongly than
10 human PDE1C, PDE2A, PDE3B, PDE4B, PDE5A, PDE7B, PDE8A, PDE10A and PDE11. IC_{50} (PDE9A) / IC_{50} (PDE1C, PDE2A, PDE3B, PDE4B, PDE5A, PDE7B, PDE8A, PDE10A and PDE11A) is preferably less than 0.2.

The selective PDE9A inhibitors are particularly suitable for improving perception,
15 concentration, learning or memory after cognitive impairments like those occurring in particular in situations/diseases/syndromes such as mild cognitive impairment, age-associated learning and memory impairments, age-associated memory losses, vascular dementia, craniocerebral trauma, stroke, dementia occurring after strokes (post stroke dementia), post-traumatic dementia, general concentration impairments,
20 concentration impairments in children with learning and memory problems, Alzheimer's disease, Lewy body dementia, dementia with degeneration of the frontal lobes, including Pick's syndrome, Parkinson's disease, progressive nuclear palsy, dementia with corticobasal degeneration, amyotrophic lateral sclerosis (ALS), Huntington's disease, multiple sclerosis, thalamic degeneration, Creutzfeld-Jacob dementia, HIV
25 dementia, schizophrenia with dementia or Korsakoff's psychosis.

The invention relates to the use of selective PDE9A inhibitors for producing medicaments for improving perception, concentration, cognitive processes, learning
and/or memory.

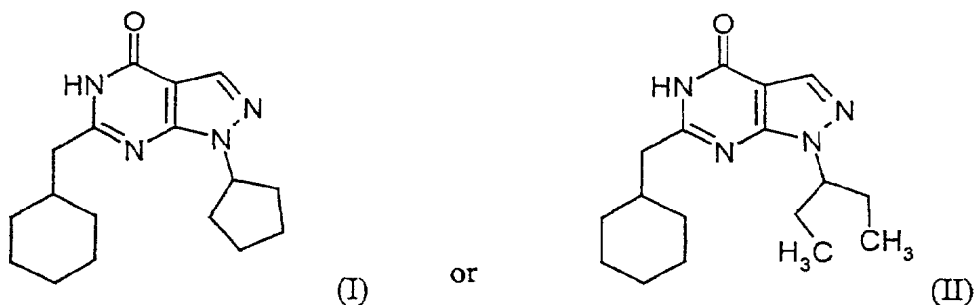
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The invention further relates to the use of selective PDE9A inhibitors for the prophylaxis and/or treatment of impairments of perception, concentration, cognitive processes, learning and/or memory.

In this connection, the impairment may be inter alia a consequence of a disorder selected from the group of dementia, stroke, craniocerebral trauma, Alzheimer's disease, Parkinson's disease, depression, or dementia with degeneration of the frontal lobes.

The invention further relates to the use of PDE9A inhibitors for the treatment of diseases of the central nervous system which are amenable to therapy through influencing the cGMP levels. Thus, the invention relates for example to the treatment of dementia, stroke, craniocerebral trauma, Alzheimer's disease, dementia with degeneration of the frontal lobes, Lewy body dementia, vascular dementia, attention deficit syndrome, impairments of attention and concentration, Parkinson's disease, schizophrenia, depression, affective disorders, psychoses, neuroses, anxiety, mania or manic depressive disorders, Pick's disease, pain and epilepsy.

The invention preferably relates to the use according to the invention of the PDE9A inhibitors of the formulae



The compounds of the formulae (I) and (II) may also be in the form of their salts, solvates or solvates of their salts. Physiologically acceptable salts are preferred for the purposes of the invention.

Physiologically acceptable salts may be salts of the compounds of the invention with inorganic or organic acids. Preference is given to salts with inorganic acids such as, for example, hydrochloric acid, hydrobromic acid, phosphoric acid or sulfuric acid,

or salts with organic carboxylic or sulfonic acids such as, for example, acetic acid, maleic acid, fumaric acid, malic acid, citric acid, tartaric acid, lactic acid, benzoic acid or methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid or naphthalenedisulfonic acid.

5

Physiologically acceptable salts may likewise be metal or ammonium salts of the compounds of the invention. Particular preference is given to alkali metal salts (e.g. sodium or potassium salts), alkaline earth metal salts (e.g. magnesium or calcium salts), and ammonium salts derived from ammonia or organic amines such as, for
10 example, ethylamine, di- or triethylamine, di- or triethanolamine, dicyclohexylamine, dimethylaminoethanol, arginine, lysine, ethylenediamine or 2-phenylethylamine.

WO 98/40384 discloses pyrazolopyrimidines which are excellent PDE1, 2 and 5 inhibitors and can be employed for the treatment of cardiovascular and cerebro-
15 vascular disorders and disorders of the urogenital system.

CH 396 924, CH 396 925, CH 396 926, CH 396 927, DE 1 147 234, DE 1 149 013, GB 937,726 describe pyrazolopyrimidines which have a coronary-dilating effect and which can be employed for the treatment of disturbances of myocardial blood flow.

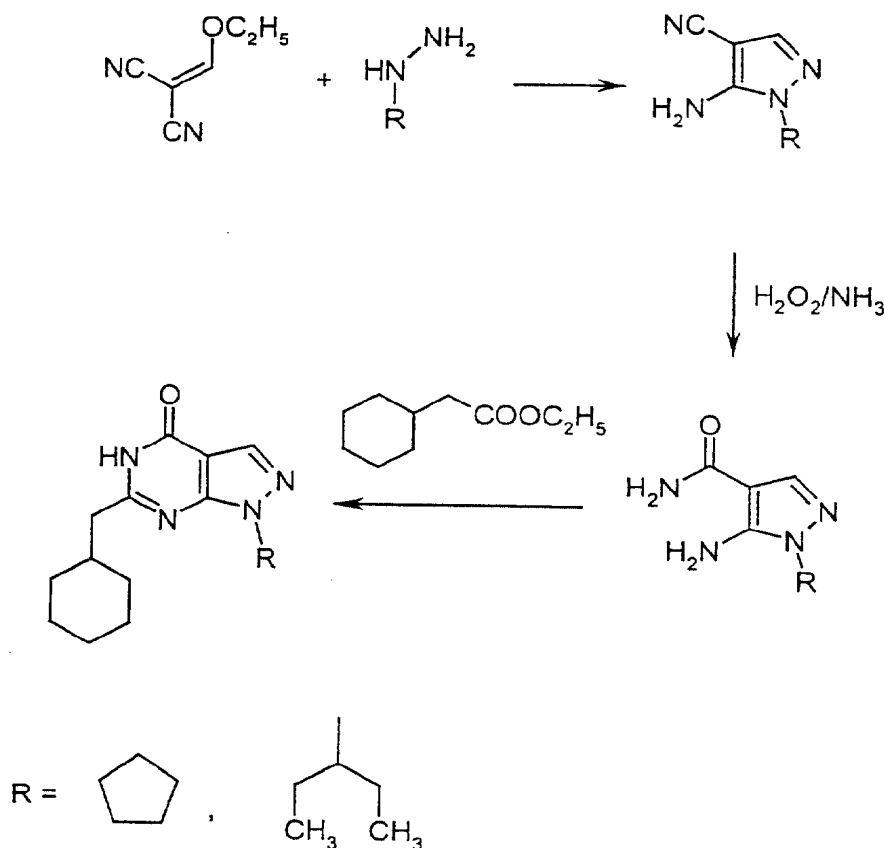
US 3,732,225 describes pyrazolopyrimidines which have an antiinflammatory and
20 blood glucose-lowering effect.

DE 2 408 906 describes styrenepyrazolopyrimidines which can be employed as antimicrobial and antiinflammatory agents for the treatment of, for example, edema.

The compounds of the invention can be prepared by the synthetic scheme described below:

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Optional subsequent reaction of the compounds of the formula (I) and (II) with the appropriate (i) solvents and/or (ii) bases or acids leads to the corresponding salts,
 5 solvates and/or solvates of the salts.

The active ingredient may have systemic and/or local effects. It can for this purpose be administered in a suitable way, such as, for example, by the oral, parenteral, pulmonary, nasal, sublingual, lingual, buccal, rectal, dermal, transdermal,
 10 conjunctival or otic route or as implant.

The active ingredient can be administered in suitable administration forms for these administration routes.

15 Administration forms suitable for oral administration are those which function according to the state of the art and deliver the active ingredient in a rapid and/or

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modified way, such as, for example, tablets (uncoated or coated tablets, for example with coatings which are resistant to gastric juice), capsules, sugar-coated tablets, granules, pellets, powders, emulsions, suspensions or solutions.

- 5 Parenteral administration can take place with avoidance of an absorption step (intravenous, intraarterial, intracardiac, intraspinal or intralumbar) or with inclusion of an absorption (intramuscular, subcutaneous, intracutaneous, percutaneous or intraperitoneal). Administration forms suitable for parenteral administration are, inter alia, injection and infusion preparations in the form of solutions, suspensions, 10 emulsions, lyophilizates or sterile powders.

Examples suitable for other administration routes are medicinal forms for inhalation (inter alia powder inhalators, nebulizers), nasal drops/solutions, sprays; tablets or capsules for lingual, sublingual or buccal administration, suppositories, preparations 15 for the ears or eyes, vaginal capsules, aqueous suspensions (lotions, shaking mixtures), lipophilic suspensions, ointments, creams, milk, pastes, foams, dusting powders or implants.

The active ingredients can be converted in a manner known per se into the stated administration forms. This takes place by using inert, non-toxic, pharmaceutically 20 suitable excipients. These include, inter alia, carriers (for example microcrystalline cellulose), solvents (e.g. liquid polyethylene glycols), emulsifiers (for example sodium dodecyl sulfate), dispersants (for example polyvinylpyrrolidone), synthetic and natural biopolymers (for example albumin), stabilizers (e.g. antioxidants such as ascorbic acid), colors (e.g. inorganic pigments such as iron oxides) and masking 25 tastes and/or odors.

It has generally proved advantageous on parenteral administration to administer amounts of about 0.001 to 30 mg/kg, preferably about 0.01 to 10 mg/kg of body weight to achieve effective results. The amount on oral administration is about 0.01 30 to 100 mg/kg, preferably about 0.1 to 30 mg/kg of body weight.

It may nevertheless be necessary to deviate from the stated amounts, in particular as

a function of body weight, administration route, individual behavior toward the active ingredient, type of preparation and time or interval over which administration takes place.

5 PDE inhibition

Recombinant PDE1C (GenBank/EMBL Accession Number: NM_005020, Loughney et al. *J. Biol. Chem.* 1996 271, 796-806), PDE2A (GenBank/EMBL Accession Number: NM_002599, Rosman et al. *Gene* 1997 191, 89-95), PDE3B (GenBank/EMBL Accession Number: NM_000922, Miki et al. *Genomics* 1996, 36, 10 476-485), PDE4B (GenBank/EMBL Accession Number: NM_002600, Obernolte et al. *Gene*. 1993, 129, 239-247), PDE5A (GenBank/EMBL Accession Number: NM_001083, Loughney et al. *Gene* 1998, 216, 139-147), PDE7B (GenBank/EMBL Accession Number: NM_018945, Hetman et al. *Proc. Natl. Acad. Sci. U.S.A.* 2000, 97, 472-476), PDE8A (GenBank/EMBL Accession Number: AF_056490, Fisher et 15 al. *Biochem. Biophys. Res. Commun.* 1998 246, 570-577), PDE9A (GenBank/EMBL accession number NM_002606, cDNA sequence see Sequence Listing, SEQ ID No: 1, Fisher et al., *J. Biol. Chem.*, 1998, 273 (25): 15559-15564), PDE10A (GenBank/EMBL Accession Number: NM_06661, Fujishige et al. *J. Biol. Chem.* 1999, 274, 18438-45), PDE11A (GenBank/EMBL Accession Number: NM_016953, 20 Fawcett et al. *Proc. Natl. Acad. Sci.* 2000, 97, 3702-3707) were expressed in Sf9 cells with the aid of the pFASTBAC baculovirus expression system (GibcoBRL). 48 h after the infection, the cells are harvested and suspended in 20 mL (per 1 L of culture) of lysis buffer (50 mM tris-HCl, pH 7.4, 50 mM NaCl, 1 mM MgCl₂, 1.5 mM EDTA, 10% glycerol plus 20 µL of protease inhibitor cocktail set III 25 [CalBiochem, La Jolla, CA USA]). The cells are treated with ultrasound at 4°C for 1 minute and subsequently centrifuged at 10000 rpm and 4°C for 30 minutes. The supernatant (PDE preparation) was collected and stored at -20°C.

The test substances are dissolved in 100% DMSO and serially diluted to determine 30 their *in vitro* effect on PDE 9A. Typically, serial dilutions from 200 µM to 1.6 µM are prepared (resulting final concentrations in the assay: 4 µM to 0.032 µM). 2 µL portions of the diluted substance solutions are introduced into the wells of microtiter plates (Isoplate; Wallac Inc., Atlanta, GA). Then 50 µL of a dilution of the PDE9A

preparation described above are added. The dilution of the PDE9A preparation is chosen so that less than 70% of the substrate is converted during the subsequent incubation (typical dilution: 1:10000; dilution buffer: 50 mM Tris/HCl pH 7.5, 8.3 mM MgCl₂, 1.7 mM EDTA, 0.2% BSA). The substrate, [8-³H] guanosine 3',5'-cyclic phosphate (1 μCi/μL; Amersham Pharmacia Biotech., Piscataway, NJ) is diluted 1:2000 with assay buffer (50 mM Tris/HCl pH 7.5, 8.3 mM MgCl₂, 1.7 mM EDTA) to a concentration of 0.0005 μCi/μL. The enzyme reaction is finally started by adding 50 μL (0.025 μCi) of the diluted substrate. The assay mixtures are incubated at room temperature for 60 min and the reaction is stopped by adding 25 μL of a PDE9A inhibitor (e.g. the inhibitor from preparation example 1, final concentration 10 μM) dissolved in assay buffer. Immediately thereafter, 25 μL of a suspension containing 18 mg/mL Yttrium Scintillation Proximity Beads (Amersham Pharmacia Biotech., Piscataway, NJ) are added. The microtiter plates are sealed with a film and left to stand at room temperature for 60 min. The plates are then measured for 30 s per well in a Microbeta scintillation counter (Wallac Inc., Atlanta, GA). IC₅₀ values are determined from the graphical plot of the substance concentration versus the percentage inhibition.

The *in vitro* effect of test substances on recombinant PDE3B, PDE4B, PDE7B, PDE8A, PDE10A and PDE11A is determined in accordance with the assay protocol described above for PDE 9A with the following adaptations: [5',8-³H] adenosine 3',5'-cyclic phosphate (1 μCi/μL; Amersham Pharmacia Biotech., Piscataway, NJ) is used as substrate. Addition of an inhibitor solution to stop the reaction is unnecessary. Instead, the incubation of substrate and PDE is followed immediately by addition of the yttrium scintillation proximity beads as described above and thus the reaction is stopped. To determine a corresponding effect on recombinant PDE1C, PDE2A and PDE5A, the protocol is additionally adapted as follows: with PDE1C, additionally 10⁻⁷ M calmodulin and 3 mM CaCl₂ are added to the reaction mixture. PDE2A is stimulated in the assay by adding 1 μM cGMP and is assayed with a BSA concentration of 0.01%. The substrate employed for PDE1C and PDE2A is [5',8-³H] adenosine 3',5'-cyclic phosphate (1 μCi/μL; Amersham Pharmacia Biotech., Piscataway, NJ), and for PDE5A is [8-³H] guanosine 3',5'-cyclic phosphate (1 μCi/μL; Amersham Pharmacia Biotech., Piscataway, NJ).

Inhibition of PDE isoenzymes by Example 2

Isoenzyme	Species	IC₅₀ [nM]
PDE1C	human	720
PDE2A	human	> 4000
PDE3B	human	> 4000
PDE4B	human	> 4000
PDE5A	human	> 4000
PDE7B	human	> 4000
PDE8A	human	> 4000
PDE9A	human	110
PDE10A	human	> 4000
PDE11	human	> 4000

- 5 The PDE9A-inhibiting effect of Example 1 can be verified experimentally by an IC₅₀ of IC₅₀ = 5 nM.

Increasing the intracellular neuronal cGMP concentration in cell cultures

- 10 PDE9A inhibitors increase the intracellular neuronal cGMP in cultivated primary cortical neurons.
- Rat embryos (embryonic day E17 - E19) were decapitated, and the heads were transferred into dissection dishes filled with dissection medium (DMEM, penicillin/streptomycin; both from Gibco). The scalp and the roof of the skull were removed, and the exposed brains were transferred into another Petri dish with dissection medium. Using a binocular microscope and two forceps, the cerebrum (cortex) was isolated and cooled to 4°C with ice. This dissection and the isolation of the cortical neurons were then carried out in accordance with a standard protocol using the Papain kit (Worthington Biochemical Corporation, Lakewood, New Jersey 08701, USA) (Huettnner et al. *J. Neurosci.* 1986, 6, 3044-3060). The mechanically isolated cortical neurons were cultivated at 150,000 cells/well in 200 µl of
- 15
- 20

- neurobasal medium/well (neurobasal; B27 supplement; 2 mM L-glutamine; in the presence of penicillin/streptomycin; all agents from Gibco) in 96-well plates (pretreated with poly-D-lysine/100 µg/ml for 30 min) under standard conditions (37°C, 5% CO₂) for 7 days. After 7 days, the medium was removed and the cells
- 5 were washed with HBSS buffer (Hank's balanced salt solution, Gibco/BRL). Subsequently, 100 µl of test substance of Example 2 dissolved in HBSS buffer (previously dissolved in 100% DMSO: 10 mM) were put on the cells. Subsequently, a further 100 µl of HBSS buffer were added, so that the final concentration of the test substance of Example 2 was as indicated in Fig. 1, and incubated at 37°C for 20 min.
- 10 The assay buffer was then completely removed. Subsequently, the cells were lysed in 200 µl of lysis buffer (cGMP kit code RPN 226; from Amersham Pharmacia Biotech.), and the cGMP concentration was measured as stated by the manufacturer. All measurements were carried out in triplicate. Statistical analysis took place using Prism Software Version 2.0 (GraphPad Software Inc., San Diego, CA USA).
- 15 Incubation of the primary neurons with Example 2 led to an increase in the cGMP content (Fig. 1).

Long-term potentiation

- Long-term potentiation is regarded as a cellular correlate of learning and memory
- 20 processes. The following method was used to determine whether PDE 9 inhibition has an influence on long-term potentiation:

- Rat hippocampi were placed at an angle of about 70 degrees to the cutting blade (chopper). 400 µm-thick slices of the hippocampus were prepared. The slices were removed from the blade using a very soft, thoroughly wetted brush (marten hair) and
- 25 transferred into a glass vessel with cold nutrient solution (124 mM NaCl, 4.9 mM KCl, 1.3 mM MgSO₄ x 7 H₂O, 2.5 mM CaCl₂²⁺ anhydrous, 1.2 mM KH₂PO₄, 25.6 mM NaHCO₃, 10 mM glucose, pH 7.4) gassed with 95% O₂/5% CO₂. During the measurement, the slices were kept in a temperature-controlled chamber under a 1-3 mm-high liquid level. The flow rate was 2.5 ml/min. The preliminary gassing
- 30 took place under a slightly elevated pressure (about 1 atm) and through a microneedle in the prechamber. The slice chamber was connected to the prechamber

in such a way that a minicirculation could be maintained. The minicirculation is driven by the 95% O₂/5% CO₂ flowing out through the microneedle. The freshly prepared hippocampus slices were adapted in the slice chamber at 33°C for at least 1 hour.

5 The stimulus level was chosen so that the focal excitatory postsynaptic potentials (fEPSP) were 30% of the maximum excitatory postsynaptic potential (EPSP). A monopolar stimulation electrode consisting of lacquered stainless steel, and a constant-current biphasic stimulus generator (AM Systems 2100) were used for local stimulation of the Schaffer collaterals (voltage: 1-5 V, pulse width of one polarity
10 0.1 ms, total pulse 0.2 ms). Glass electrodes (borosilicate glass with filament, 1-5 MOhm, diameter: 1.5 mm, tip diameter: 3-20 µm), filled with normal nutrient solution, were used to record the excitatory postsynaptic potentials (fEPSP) from the stratum radiatum. The field potentials were measured versus a chlorinated silver
15 amplifier. The field potentials were filtered through a low-pass filter (5 kHz). The slope of the fEPSPs (fEPSP slope) was determined for the statistical analysis of the experiments. The recording, analysis and control of the experiment took place with the aid of a software program (PWIN) which was developed in the Department of Neurophysiology of the Leibniz Neurobiology Institute, Magdeburg. The formation
20 of the average fEPSP slopes at the respective time points and construction of the diagrams took place with the aid of the EXCEL software, with automatic data recording by an appropriate macro.

In the control experiments, the baseline synaptically transmission was initially recorded for 60 - 120 minutes. Subsequently, two double pulses were administered
25 four times at an interval of 200 ms, with an interpulse interval of 10 ms for the double pulses and a width of 0.2 ms for the individual pulses (weak tetanus) (time = 0 minutes in Figure 2). The resulting potentiation of the EPSPs was recorded for at least 60 minutes. Example 2 was flushed in for 10 minutes before up to 30 minutes after the stimulation in the experiment depicted.

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Superfusion of the hippocampus slices with a 10 µM solution of Example 2 led to a significant increase in the LTP (Figure 2).

Social recognition test:

The social recognition test is a learning and memory test. It measures the ability of rats to distinguish between known and unknown members of the same species. This test is therefore suitable for examining the learning- or memory-improving effect of test substances.

Adult rats housed in groups were placed singly in test cages 30 min before the start of the test. Four minutes before the start of the test, the test animal was put in an observation box. After this adaptation time, a juvenile animal was put in with the test animal and the absolute time for which the adult animal inspected the young one was measured for 2 min (trial 1). All behaviors clearly directed at the young animal were measured, i.e. anogenital inspection, pursuit and grooming, during which the old animal was no further than 1 cm from the young animal. The juvenile was then removed, and the adult was treated with Example 2 or vehicle and subsequently returned to its own cage. The test was repeated after a retention time of 24 hours (trial 2). A diminished social interaction time compared with trial 1 would indicate that the adult rat remembers the young animal.

The adult animals received intraperitoneal injections directly following trial 1 either with vehicle (10% ethanol, 20% Solutol, 70% physiological saline) or 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg or 3.0 mg/kg of Example 2 dissolved in 10% ethanol, 20% Solutol, 70% physiological saline. Vehicle-treated rats showed no reduction in the social interaction time in trial 2 compared with trial 1. They had consequently forgotten that they had already had contact with the young animal. Surprisingly, the social interaction time in the second run after treatment Example 2 was significantly reduced compared with those treated with vehicle. This means that the substance-treated rats have remembered the juvenile animal and thus Example 2 displayed an improving effect on learning and memory.

Abbreviations used:

DMSO	dimethyl sulfoxide
equiv.	equivalent(s)

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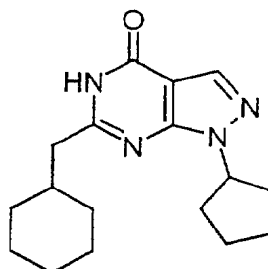
- 15 -

ESI	electrospray ionization (in MS)
HPLC	high pressure, high performance liquid chromatography
MS	mass spectroscopy
m.p.	melting point
5 NMR	nuclear magnetic resonance spectroscopy

Exemplary embodiments:

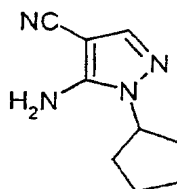
Example 1

6-(Cyclohexylmethyl)-1-cyclopentyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one



Stage 1a)

5-Amino-1-cyclopentyl-1H-pyrazole-4-carbonitrile



Cyclopentylhydrazine (6.5 g, 64.9 mmol) is slowly added to a solution of ethoxymethylenemalononitrile (7.93 g, 64.9 mmol) in 100 ml of methanol at room temperature under argon, subsequently heated under reflux for 3 h and then stirred at room temperature overnight. The solvent is stripped off in a rotary evaporator and the residue is stirred with diethyl ether. The solid is filtered off with suction, washed with diethyl ether and dried under high vacuum.

Yield: 7.24 g (63% of theory)

MS (ESI): $m/z = 177 (M+H)^+$

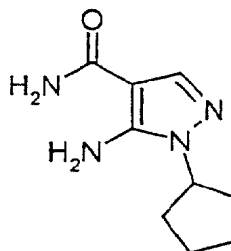
$^1\text{H-NMR}$ (200 MHz CDCl_3): $\delta = 7.5$ (s, 1H), 4.45 (br. s, 2H), 4.35 (m, 1H), 2.2-1.55

(m, 6H) ppm.

Stage 1b)

5-Amino-1-cyclopentyl-1H-pyrazole-4-carboxamide

5



85 ml of 30% strength hydrogen peroxide solution are added to a solution of 5-amino-1-cyclopentyl-1H-pyrazole-4-carbonitrile (6.74 g, 38.3 mmol) in a mixture of
 10 300 ml of ethanol and 371 ml of concentrated aqueous ammonia solution at room temperature and stirred at room temperature overnight. The nonaqueous solvents are then stripped off in a rotary evaporator. The product precipitates as solid from the remaining mixture and is filtered off with suction, washed with diethyl ether and dried under high vacuum.

15 Yield: 5.31 g (71% of theory)

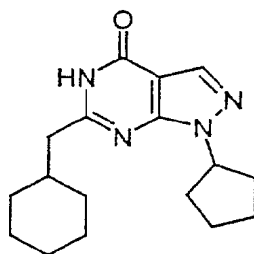
MS(ESI): $m/z = 195 (M+H)^+$

$^1\text{H-NMR}$ (200 MHz, CDCl_3): $\delta = 7.5$ (s, 1H), 5.67-4.8 (broad, 4H), 4.35 (m, 1H), 2.2-1.55 (m, 8H) ppm.

20 Stage 1c)

6-(Cyclohexylmethyl)-1-cyclopentyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one

- 18 -



Under argon, 75 mg (0.39 mmol) of 5-amino-1-cyclopentyl-1H-pyrazole-4-carbox-
 amide and 183 mg (1.16 mmol, 3 equiv.) of methyl cyclohexylacetate are introduced
 5 into 1.5 ml of absolute ethanol. At 0°C, 54 mg of sodium hydride (60% dispersion in
 mineral oil; 1.35 mmol, 3.5 equiv.) are slowly added in a countercurrent of argon.
 The resulting mixture is slowly heated and stirred under reflux for 18 h. For workup,
 20 ml of water are added, and the mixture is extracted several times with ethyl
 acetate. The combined organic phases are dried over sodium sulfate and concentrated
 10 in vacuo. The crude product is purified by preparative HPLC.

Yield: 36 mg (31% of theory)

MS (ESI): $m/z = 301$ ($M+H$)⁺

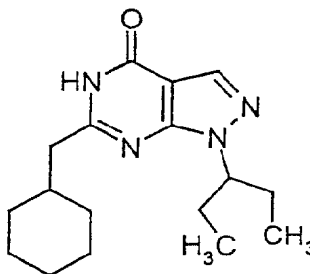
m.p.: 147°C

¹H-NMR (300 MHz, DMSO-d₆): $\delta = 11.95$ (s, 1H), 8.0 (s, 1H), 5.1 (m, 1H), 2.5 (d,
 15 2H), 2.15-1.75 (m, 7H), 1.75-1.55 (m, 7H), 1.3-0.9 (m, 5H) ppm.

Example 2

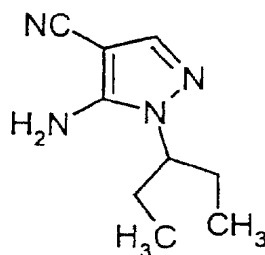
**6-(Cyclohexylmethyl)-1-(1-ethylpropyl)-1,5-dihydro-4H-pyrazolo[3,4-d]-
 pyrimidin-4-one**

20



Stage 2a)

5-Amino-1-(1-ethylpropyl)-1H-pyrazole-4-carbonitrile



5

Preparation takes place in analogy to the method for Example 1 / stage 1a).

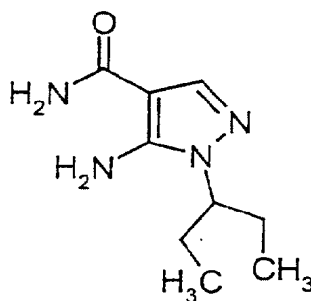
MS (ESI): $m/z = 179$ (M+H)⁺

¹H-NMR (300 MHz, DMSO-d₆): $\delta = 7.55$ (s, 1H), 6.45 (s, 2H), 4.0 (m, 1H), 1.8-1.55

10 (m, 4H), 0.65 (t, 6H) ppm.

Stage 2b)

5-Amino-1-(1-ethylpropyl)-1H-pyrazole-4-carboxamide



15

Preparation takes place in analogy to the method for Example 1 / stage 1b).

MS (ESI): $m/z = 197$ (M+H)⁺

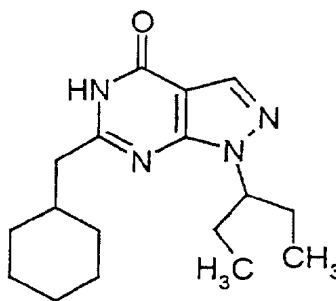
¹H-NMR (300 MHz, DMSO-d₆): $\delta = 7.65$ (s, 1H), 6.9 (br. s, 2H), 6.1 (s, 2H), 3.9 (m,

20 1H), 1.85-1.6 (m, 4H), 0.7 (t, 6H) ppm.

Stage 2c)

6-(Cyclohexylmethyl)-1-(1-ethylpropyl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one

5



The product is obtained in analogy to Example 1 / stage 1c) starting from 200 mg (1.02 mmol) of 5-amino-1-(1-ethylpropyl)-1H-pyrazole-4-carboxamide and 482 mg (3.06 mmol) of methyl cyclohexylacetate.

Yield: 146 mg (47% of theory)

MS (ESI): $m/z = 303$ (M+H)⁺

m.p.: 122°C

¹H-NMR (200 MHz, DMSO-d₆): $\delta = 12.0$ (s, 1H), 8.0 (s, 1H), 4.45 (m, 1H), 2.5 (m, 2H), 2.0-1.5 (m, 10H), 1.4-0.9 (m, 5H), 0.6 (t, 6H, $J = 7.5$ Hz) ppm.

- 1 -

SEQUENCE LISTING

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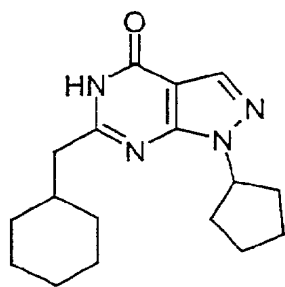
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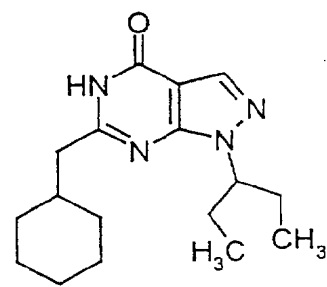
Claims

1. The use of selective PDE9A inhibitors for producing medicaments for improving perception, concentration, cognitive processes, learning and/or memory.
5
2. The use as claimed in claim 1 for the prophylaxis and/or treatment of impairments of perception, concentration, cognitive processes, learning and/or memory.
10
3. The use as claimed in claim 2, where the impairment is a consequence of a disorder selected from the group consisting of dementia, stroke, craniocerebral trauma, Alzheimer's disease, Parkinson's disease, depression, or dementia with degeneration of the frontal lobes.
15
4. The use of PDE9A inhibitors for producing medicaments for the treatment and/or prophylaxis of diseases of the central nervous system which are amenable to therapy through influencing the cGMP levels.
20
5. The use as claimed in claim 4, where the disease is selected from a group of diseases consisting of dementia, stroke, craniocerebral trauma, Alzheimer's disease, dementia with degeneration of the frontal lobes, Lewy body dementia, vascular dementia, attention deficit syndrome, impairments of attention and concentration, Parkinson's disease, schizophrenia, depression, affective disorders, psychoses, neuroses, anxiety, mania or manic depressive disorders, Pick's disease, pain and epilepsy.
25
6. The use as claimed in any of claims 1 to 5, where the selective PDE9A inhibitor is a compound of the formula
30



(I)

or



(II)

and the salts, solvates and solvates of the salts thereof.

Fig. 1

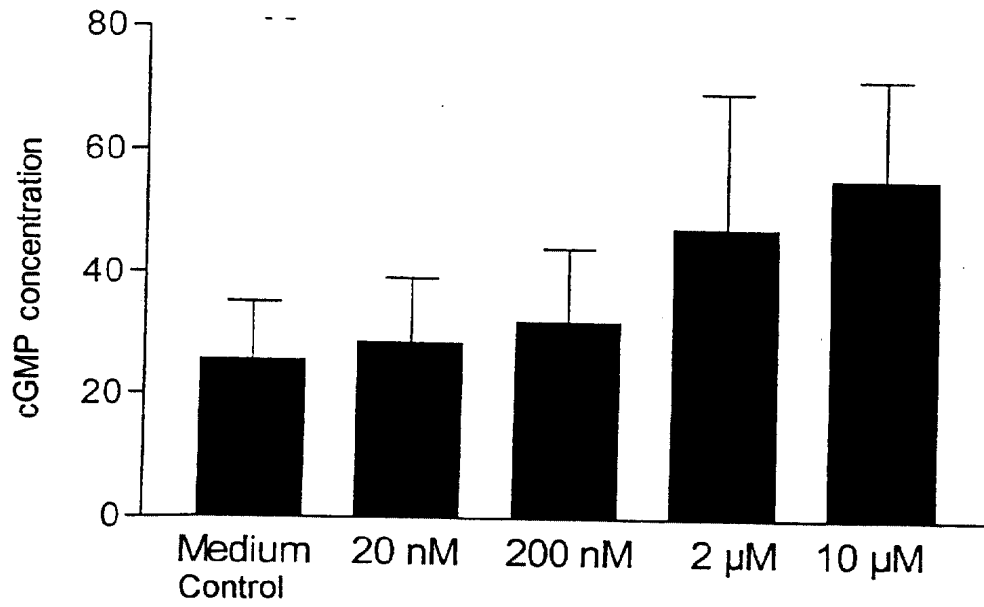


Fig. 2

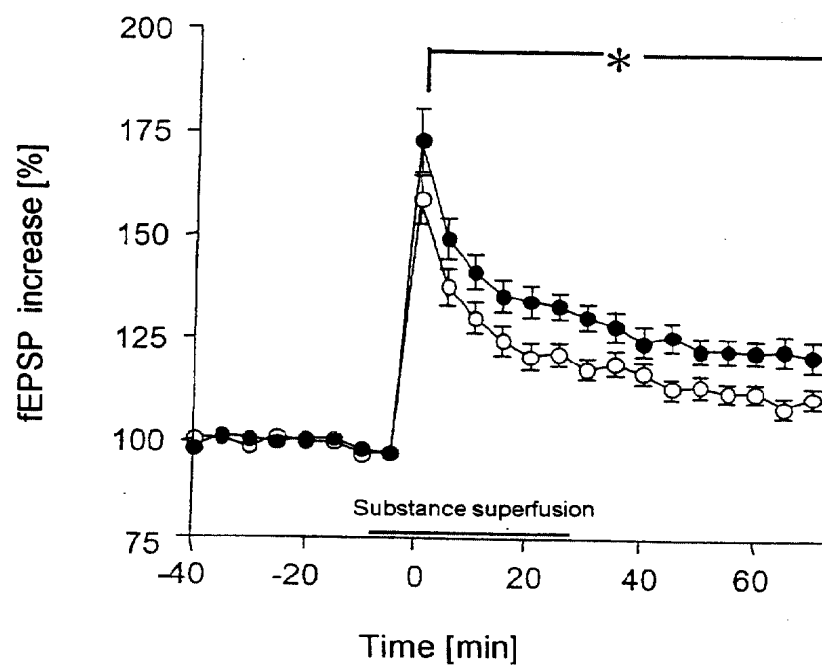


Fig. 3

